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# RNA sequencing reveals that *Prx II* gene knockout can down-regulate the allograft rejection of dermal mesenchymal stem cells

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## Abstract

In this study, we used RNA sequencing (RNA-seq) to analyze and compare bulk cell samples from wild-type (WT) dermal mesenchymal stem cells (DMSCs) (n = 3) and *Prx II* knockout DMSCs (n = 3). The purpose of the study was to elucidate the role of *Prx II* on allogeneic immune rejection of transplanted DMSCs. The results revealed differential expression of 472 genes (176 up-regulated and 296 down-regulated;  $p \leq 0.05$ ) between the *PrxII*<sup>+/+</sup> (WT) and *PrxII*<sup>-/-</sup> sample groups. When highly regulated genes were categorized according to the Gene Ontology (GO) molecular function classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, the *PrxII*<sup>-/-</sup> samples showed a robust downward trend in allograft rejection. The study identified 43 all immunologically rejected differentially expressed genes, of which 41 showed lower expression in the *PrxII*<sup>-/-</sup> vs. *PrxII*<sup>+/+</sup> (WT) samples. These findings suggest that *Prx II* gene knockout may down-regulate the allograft rejection that occurs during DMSCs transplantation and improve the survival rate of DMSCs in the host. This study provides a new perspective on the clinical treatment of stem cell transplantation.

**Keywords:** Allograft rejection, Dermal mesenchymal stem cells, Peroxiredoxin II, RNA sequencing

## Introduction

Mesenchymal stem cells (MSCs) are pluripotent stem cells that exist in many tissues and can differentiate into several cell types [1, 2]. Therefore, they are widely used in transplantation and tissue regeneration. Dermal

mesenchymal stem cells (DMSCs) are derived from skin and offer several advantages such as abundant source, easy collection, and low infection rate with pathogenic microorganisms, low immunogenicity, and secretion of a large number of cytokines. Consequently, DMSCs have broad application prospects in the clinical treatment of many diseases and the potential to regenerate organs and prolong life.

A large number of preclinical animal experiments have shown that MSCs can be implanted to treat myocardial infarction [3], spinal cord injury [4], diabetic foot [5] and other diseases. However, transplantation rejection is still an inevitable problem in the practical application of allogeneic mesenchymal stem cells and their derivatives. The major cause of immune rejection is the activation of T lymphocytes by the major histocompatibility complex (MHC), which results in a specific immune response

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[6, 7] with serious side effects that can render the treatment ineffective. Strategies to reduce organ rejection and improve the survival rate after transplantation will be crucial in the development of transplantation medicine.

The immune response includes innate immunity and acquired immunity, with the latter divided into humoral and cellular immune responses. The main contributor to immune rejection is the cellular immunity. The main function of the MHC is to activate T lymphocytes to produce specific immune responses. Cell surface antigens encoded by complex gene groups play a leading role in immune rejection [8], and MHC molecules are found on the cell surface of all vertebrates. Because T cell receptors recognize only a small fraction of antigenic molecule determinants, the T cell immune response requires the processing and presentation of antigens by antigen-presenting cells. There are two pathways involved in the process [9, 10]: the MHC-I and the MHC-II class pathways. In the MHC-I pathway, endogenous antigens in the cytoplasm form complexes with MHC-I molecules, which are transported to the cell surface for recognition by CD8<sup>+</sup> T cells. In the MHC-II pathway, exogenous antigens form complexes with MHC-II molecules via the endoplasmic lysosomal pathway, which are then transported to the cell surface for recognition by CD4<sup>+</sup> T cells. In addition to the cellular immune response, the humoral immune response and various regulatory factors also play an important role in the immune response.

Chemokines are a large cytokine superfamily composed of small secreted proteins. In recent years, chemokines have been found to play an important role in transplantation rejection, studies have shown that the role of chemokines in transplantation rejection is mainly related to the recruitment of T-lymphocytes [11]. It has been shown that the expression of CXCL9/MIG in allografted skin leads to complete rejection within a few days and that neutralization of CXCL9/MIG could inhibit the infiltration of T cells and delay rejection [12, 13]. Similarly, cardiac allograft rejection has a time pattern of chemokine expression. Both CCL3 and CCL4 are expressed in the late phase of transplantation rejection, whereas CXCL10 and CXCL9 are highly expressed in the early and late phases of allograft rejection [14]. These patterns are also seen in human heart transplantation, indicating that chemokines do indeed play a key role in allograft rejection.

RNA sequencing (RNA-seq) using next-generation sequencing (NGS) platforms greatly improves the analysis of whole transcriptomes, allowing full annotation and quantification of a large number of genes in one run. This technique can detect known and non-characteristic transcripts and provide information about alternative and new splicing events [15]. Using the RNA-Seq

technology, we analyzed the whole transcriptome of PrxII<sup>+/+</sup> and PrxII<sup>-/-</sup> DMSCs samples. We found that a group of cytokines and chemokines was involved in the immune rejection process of both samples. Compared to the WT, the expression of most of these cytokines and chemokines was down-regulated in the Homo samples. These results suggest that reducing *Prx II* expression may be an effective way to reduce DMSCs rejection.

## Materials and methods

### Chemicals

Dulbecco's Modified Eagle's Medium (DMEM) and Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) were purchased from Gibco (Gibco, CA, USA), fetal bovine serum (FBS) were purchased from Hyclone (General Electric Healthcare Life Sciences, Mississauga, Canada), penicillin streptomycin (P/S) was purchased from solarbio (Solarbio life sciences, Beijing, China), TRIzol was purchased from Sigma (Sigma, St. Louis, MO, USA).

### Quality control chart

Clean reads were obtained after removal of the adaptor, the low-quality reads, and the ribosomal sequences (Fig. 1). If the probability of a read's error at a certain position is  $p=0.01$ , the y-coordinate value on the graph corresponds to  $-10 * \log_{10}(p)=20$ , red represents the median, yellow represents the 25–75% range, tentacle is the 10–90% range, and blue line is the average.

### Cell culture

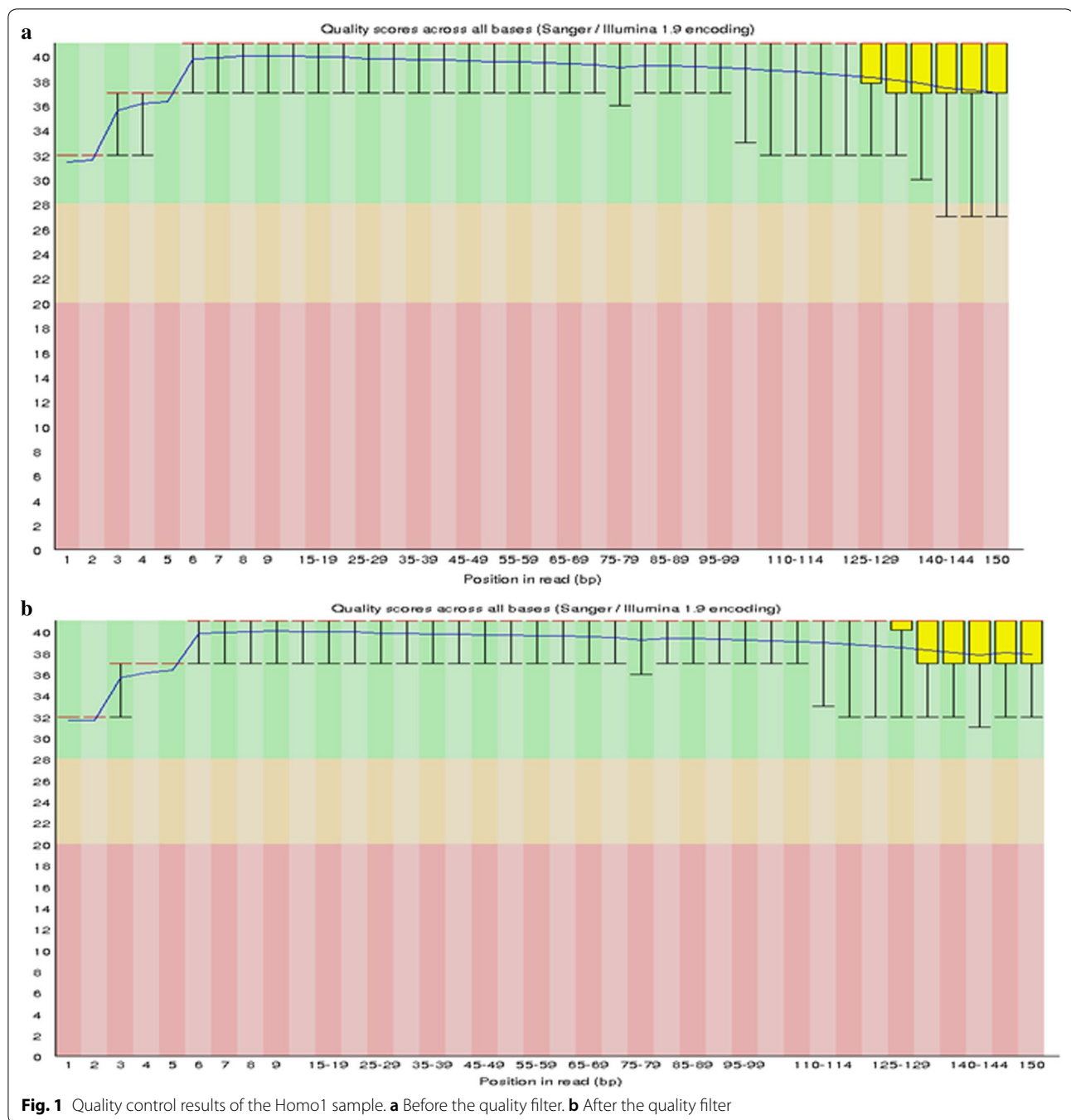
DMSCs were extracted from the skin of 129/SvJ mice. Cell cultures were maintained in DMEM/F12 medium supplemented with 10% (v/v) FBS and antibiotics (100 U/ml of penicillin and 100 µg/ml of streptomycin), and incubated at 37 °C and 5% CO<sub>2</sub>. Cells were sub-cultured once every 2 days.

### Sample preparation

The six samples (WT 1-3 and Homo 1-3) were lysed from 129/SvJ mice using TRIzol Reagent according to the manufacturer's manual (Invitrogen, Carlsbad, CA), recycled to independent 1.5 ml tubes and sent to the Gminix company for total RNA extraction and RNA sequencing.

### RNA sequencing

The resulting RNA was randomly broken into fragments and reverse transcribed using random primers containing Poly (T) oligonucleotides and reverse transcriptase. We used Poly (T) oligonucleotides for reverse transcription PCR to extract all mature eukaryotic mRNAs, which contain Poly (A) tails as a post-transcriptional modification. Then, the ends of the resulting cDNA fragments



were repaired and the adapter was connected to obtain the cDNA used for sequencing. Lastly, a new generation of high-throughput sequencing was used.

**Identification of differentially expressed genes**

To study the differences between the two sample groups (PrxII<sup>+/+</sup> and PrxII<sup>-/-</sup>), gene expression in both groups was compared using DESeq2. In this analysis, a significant *p*-value and a fold-change (FC) between genes were

obtained. The screening conditions were *p* < 0.05 and FC cut-offs of > 1.2 and < 0.83333, and yielded the identification of 472 differentially expressed genes between the two sample groups.

**Reconstruction of co-expression network**

The pathway analysis used was based on the KEGG database. Fisher exact test and Chi square test were used for differentially expressed genes. Then significance analysis

is carried out for the pathway participated by the target gene, and the pathway with significance is screened following to  $p < 0.05$ . According to the significance level ( $p$ -value) of each differential gene pathway and the number of differential genes contained in the down-regulated significant differential gene pathway, a bubble chart was plotted for the top gene pathways that were significantly down-regulated ( $p < 0.05$ ). For the construction of a co-expression network, the genes from the bubble chart were analyzed using 'ClueGo' from the Cytoscape v3.2 [16].

## Results

### Sample correlation analysis and principal component analysis

To determine whether the gene expression profiles presented different correlations between the two sample groups, we analyzed the RNA-seq data of the WT and Homo samples using the Pearson correlation coefficient (Fig. 2a). The results showed that both sample groups were very similar. Similar gene expression was also supported by the principal-component analysis (Fig. 2b).

### Gene expression profiling of samples by RNA sequencing analysis

In this study, I analyzed the differentially expressed (DE) genes between the WT and Homo sample groups. Using  $p < 0.05$  and  $FC > 1.2$  or  $< 0.83333$  as the screening

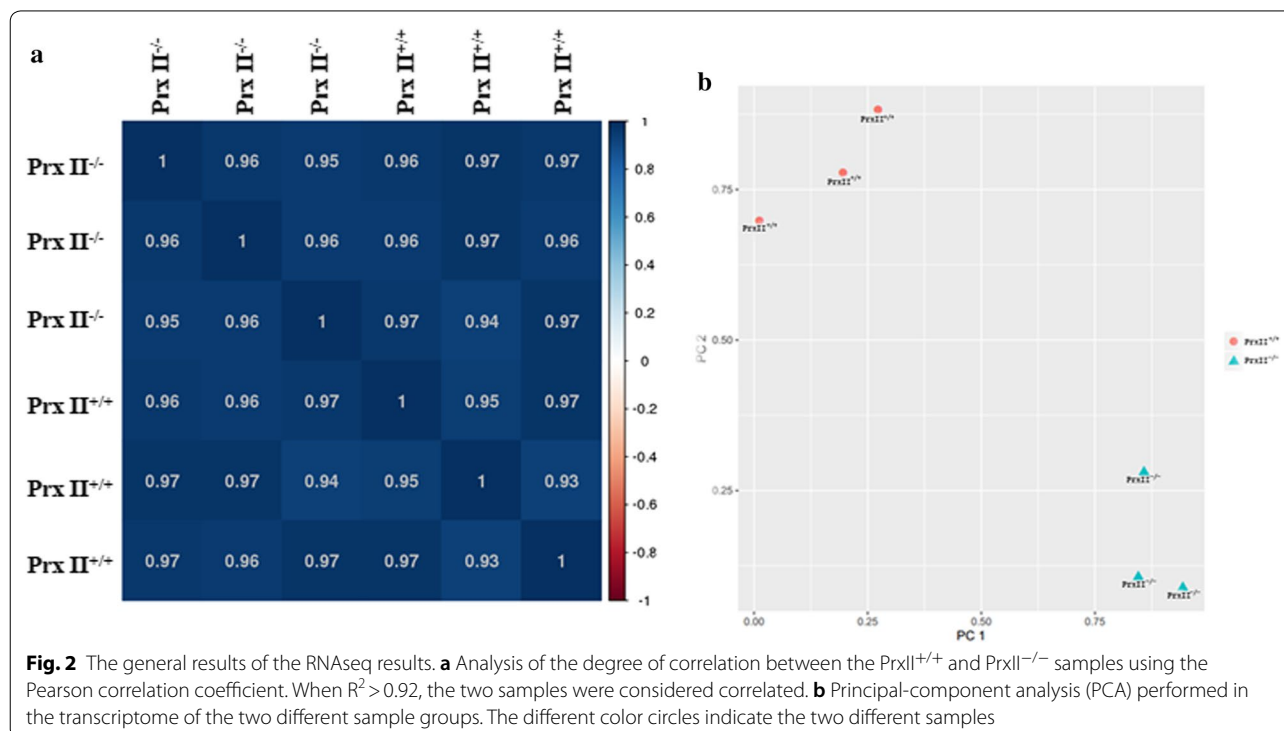
conditions, we identified 472 DE genes (176 up-regulated and 296 down-regulated) between the WT and Homo sample groups (Fig. 3a). All DE genes were plotted in a heat map (Fig. 3b).

### Functional enrichment analysis of highly regulated genes

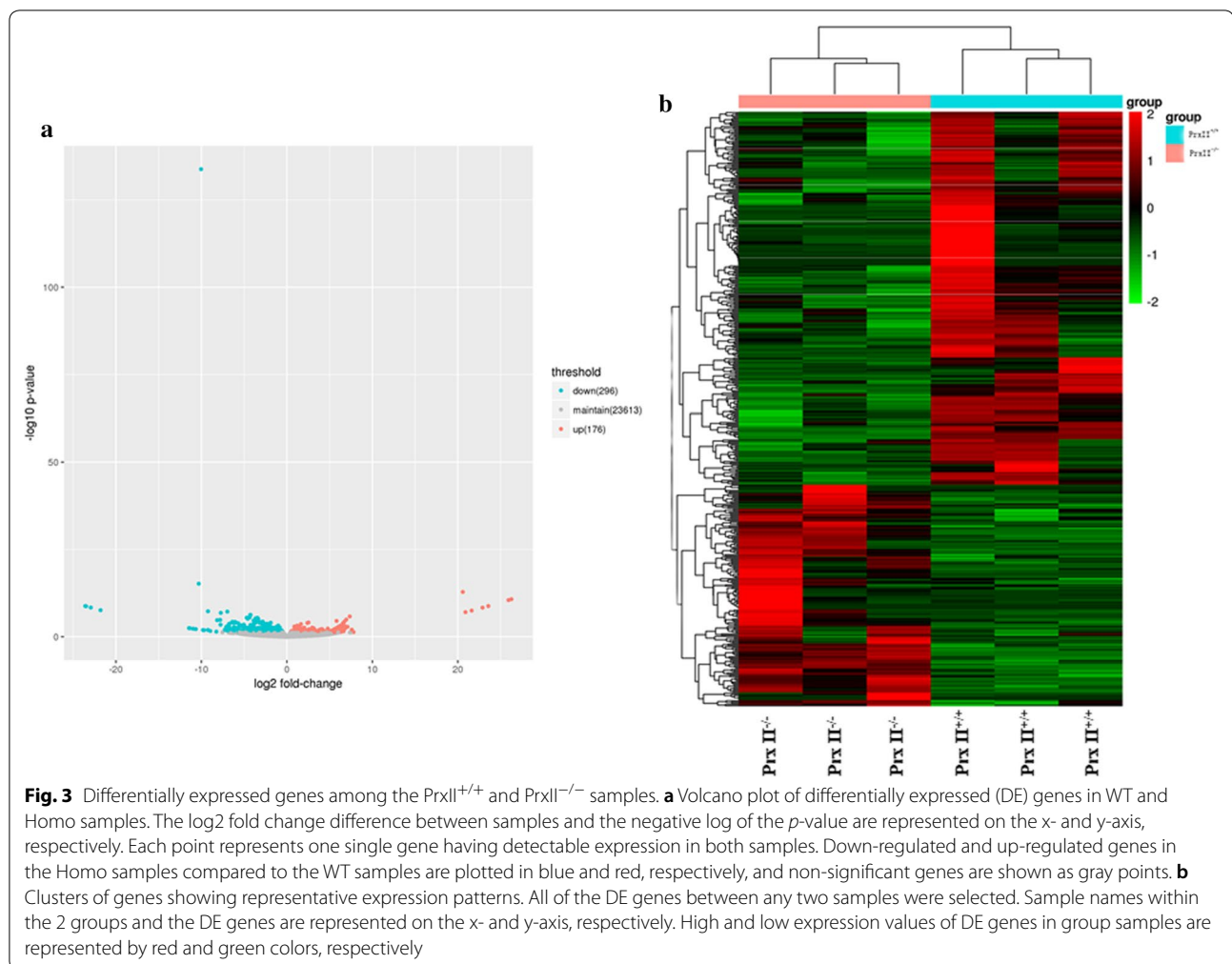
To investigate the biological role of the DE genes at the transcriptional level, we categorized the 472 DE genes into enriched categories according to the GO molecular function classification and the KEGG pathway analysis.

There were 20 top GO categories that were significantly enriched with a  $p < 0.05$ , which included the following: TAP binding; peptide antigen binding; T cell receptor binding; antigen binding. The complete list of genes included in each category is shown in Table 1. The results show that most of the DE genes were related to immune function. We found both up-regulated and down-regulated genes within the GO enriched categories (Table 1). In this respect, we found that many genes encoding potentially secreted proteins are down-regulated in the Homo sample, whereas the *Cx3cl1* and *Cxcr6* genes were up-regulated.

To evaluate the enrichment in signaling transduction pathways, we analyzed the 472 genes with the KEGG pathway analysis. In addition, the top 24 gene pathways that were significantly ( $p < 0.05$ ) down-regulated were plotted in a bubble chart (Fig. 4, Table 2). These results suggest that the most enriched pathways were



**Fig. 2** The general results of the RNAseq results. **a** Analysis of the degree of correlation between the Prxl<sup>+/+</sup> and Prxl<sup>-/-</sup> samples using the Pearson correlation coefficient. When  $R^2 > 0.92$ , the two samples were considered correlated. **b** Principal-component analysis (PCA) performed in the transcriptome of the two different sample groups. The different color circles indicate the two different samples



related to antigen processing and presentation, which are known to be associated with allograft rejection.

#### Network reconstruction using down-regulated genes in the bubble chart

In the first analysis, the WT samples were compared to the Homo samples. This analysis found five pathways associated with allograft rejection that consisted of down-regulated genes in the bubble chart (Table 3). These DE genes were used to reconstruct the co-expression network of high co-expression genes. We used the ClueGo in the Cytoscape (v3.2.0) to analyze the relationship between the pathways and the DE genes, using a parameter of  $p < 0.05$ . The results show that the expression of allograft rejection-associated genes (41/43) in the Homo samples was lower than in the WT samples (Fig. 5).

#### Discussion

MSCs are pluripotent cells that exist in a variety of tissues (bone marrow, cord blood and cord tissue, placental tissue, etc.) [17]. MSCs have the potential for multi-directional differentiation, and a unique cytokine secretion function. These properties make them ideal seed cells for gene and cell therapy. They have a broad application prospect in the treatment of nervous system injury [18], premature ovarian failure [19], bone defect [20] and other diseases. It is believed that MSCs used in the treatment of several diseases can replace and replenish damaged cells through their own proliferation and differentiation [21]. Recent studies have shown that transplanted MSCs have a relatively low survival rate in the damaged area and rarely differentiate into other types of cells. However, the transplanted MSCs were able to repair the site of injury even though it was remote from the actual injury, suggesting that the stem cells could exert their therapeutic

**Table 1** Go analysis of differentially expressed genes in *PrxII*<sup>+/+</sup> (WT) and *PrxII*<sup>-/-</sup>

GO ID	GO term	Genes	p-value
GO:0046977	TAP binding	Tapbp/Tap2/H2-Q4/Tap1/H2-Q7/H2-K1/H2-Q6/H2-D1/H2-Q1	5.13E-07
GO:0042605	peptide antigen binding	Tap2/H2-Q4/Tap1/H2-T24/H2-Q7/H2-K1/H2-T23/H2-Q6/H2-D1/H2-Q1	1.70E-11
GO:0042608	T cell receptor binding	H2-Q4/H2-Q7/H2-K1/H2-T23/H2-Q6/H2-D1/H2-Q1	1.05E-10
GO:0003823	Antigen binding	Cd40/Tap2/H2-Q4/Tap1/H2-T24/H2-Q7/H2-K1/H2-T23/H2-Q6/H2-D1/H2-Q1	8.62E-06
GO:0042379	Chemokine receptor binding	Ccl8/Stat1/Cxcl9/Cxcl10/Ccl5/Nes/Cx3cl1	1.96E-04
GO:0042288	MHC class I protein binding	Tap2/Tap1/H2-T23	5.19E-04
GO:0008009	Chemokine activity	Ccl8/Cxcl9/Cxcl10/Ccl5	7.01E-04
GO:0042277	Peptide binding	Tap2/H2-Q4/Tap1/H2-T24/H2-Q7/H2-K1/H2-T23/H2-Q6/H2-D1/H2-Q1	9.14E-04
GO:0048248	CXCR3 chemokine receptor binding	Cxcl9/Cxcl10	9.99E-04
GO:0004298	Threonine-type endopeptidase activity	Psmb8/Psmb10/Psmb9	1.19E-03
GO:0070003	Threonine-type peptidase activity	Psmb8/Psmb10/Psmb9	1.19E-03
GO:0044389	Ubiquitin-like protein ligase binding	Daxx/Cd40/Parp9/Stat1/Tnfrsf1b/Pml/Txnip/Stat2/Ddx58/Dtx3l	1.79E-03
GO:0031730	CCR5 chemokine receptor binding	Stat1/Ccl5	2.07E-03
GO:0042287	MHC protein binding	Tap2/Tap1/H2-T23	4.46E-03
GO:0048020	CCR chemokine receptor binding	Ccl8/Stat1/Ccl5	5.27E-03
GO:0045236	CXCR chemokine receptor binding	Cxcl9/Cxcl10/Cxcr6	7.39E-03
GO:1904680	Peptide transmembrane transporter activity	Tap2/Tap1	1.89E-02
GO:0016504	Peptidase activator activity	Psme1/Pycard	4.38E-02
GO:0004252	Serine-type endopeptidase activity	C2/C1s1/C1ra/C1s2/Cfb	4.43E-02
GO:0004866	Endopeptidase inhibitor activity	Pttg1/Serping1/C3/Nlrc4/Bst2	4.51E-02

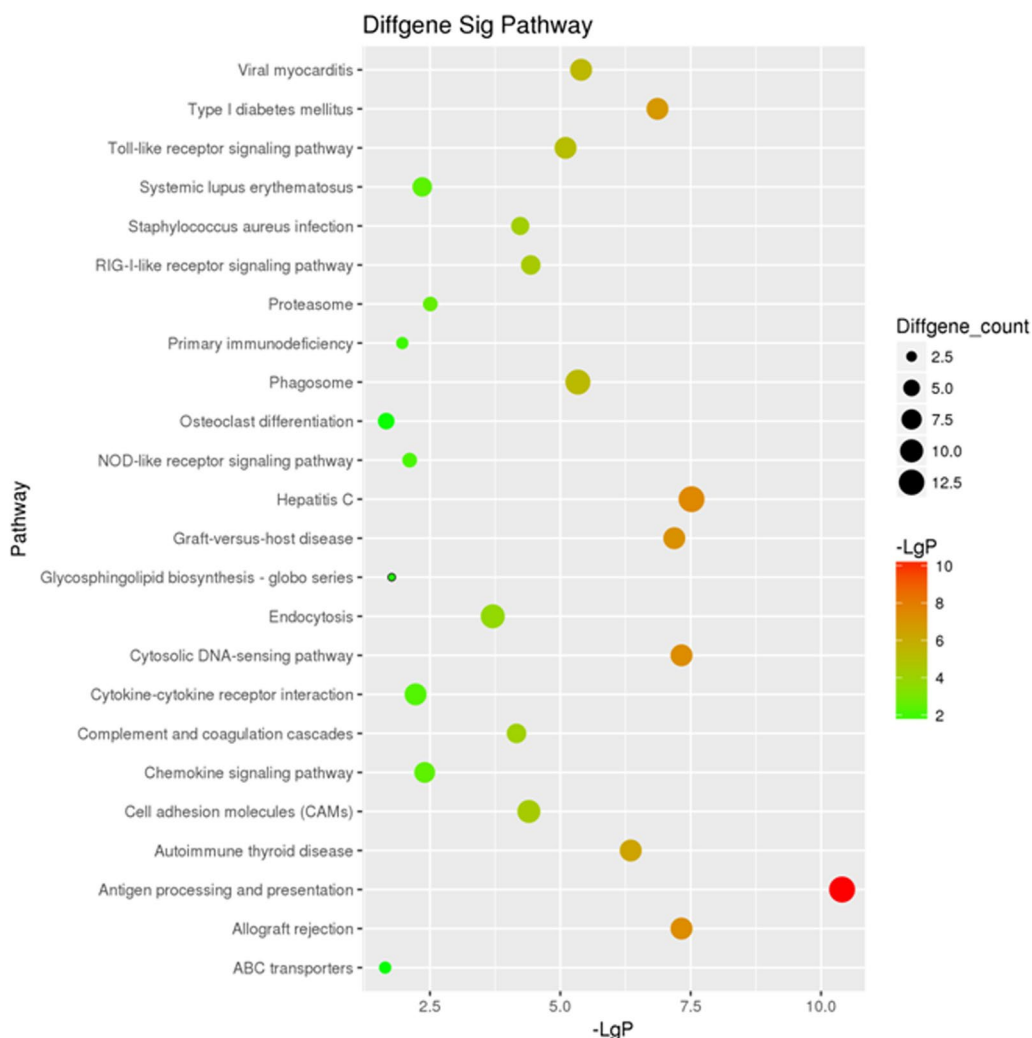
effects in a paracrine manner [22, 23]. Regardless of the therapeutic mechanism, the main problem after MSCs transplantation is still the host immune rejection.

Peroxiredoxins (Prxs) are a highly conserved superfamily of peroxidases. Prxs play an important role in gene expression, internal immunity and inflammation-related physiological reactions such as tissue repair, parasite infection and tumor formation [24]. *Prx II* is a member of the Prx family with a molecular weight of about 22 kDa, which is localized in the cytoplasm and expressed in a variety of human tissues. *Prx II* can inhibit the replication of HIV in human T cells [25, 26]. Moreover, the *Prx II* oligomer (300–400 kDa) is an active natural killer (NK) cell enhancer (NKEF-B) [27, 28]. Treatment of *Prx II* with dithiothreitol inhibited its oligomerization process and eliminated virtually all its NK cell enhancing activity. Furthermore, the sulfhydryl group of *Prx II* was blocked by *n*-ethylmaleimide. This activity was completely lost after the formation of dimer and oligomer was inhibited [29]. In summary, these results suggests a potential link between *Prx II* and the immune system.

Immune rejection is a process by which the body destroys the grafts (allogeneic cells, tissues, or organs) through a specific immune response. In general, after transplantation, the recipient can recognize and respond to the graft antigen, and the immune cells in the graft can also recognize and respond to the recipient antigen

tissue. The main function of the MHC is to activate T lymphocytes to produce specific immune responses. Because the T cell receptor (TCR) can only recognize a small part of the determinant of antigen molecules, the immune response process needs the processing and presentation of antigens by antigen-presenting cells (APCs). In addition, T cell recruitment and the complement activation pathway also play important roles in the immune response.

The present study used bulk cell samples for transcriptome sequencing. The results show 472 differentially expressed (DE) genes in the two samples, with down-regulated genes more prominent than up-regulated genes. Therefore, we carried out GO and KEGG analyses on the down-regulated genes. In the down-regulated signaling pathways, we found five signaling pathways related to immune rejection: allograft rejection; antigen processing and presentation; proteasome; the chemokine signaling pathway; and complement and coagulation cascades. In this study, we found that after knockout of *Prx II*, the expression of *Psmb8*, *Psmb10*, *Psmb9*, *Psme1*, *Tapbp*, *Tap2*, *Tap1*, *B2m*, *H2-T24*, *H2-Q7*, *H2-K1*, *H2-T23*, *H2-Q6*, *H2-D1*, *H2-T10*, and *H2-Q1* genes were down-regulated in DMSCs. Consequently, antigen processing, transport and MHC-I molecular assembly, and formation and presentation of peptide-MHC-I complex, were all inhibited. Moreover, the expression of *Ccl8*, *Stat1*,



**Fig. 4** Functional enrichment analysis of the down-regulated DE genes. **a** Bubble chart of KEGG pathway enrichment analyses for down-regulated DE genes. Different bubble color represents the negative log of the  $p$ -value. Red indicates highly statistically significant. ( $p < 0.05$ ) Bubble size indicates gene numbers enriched in a pathway

*Cxcl9*, *Rasgrp2*, *Cxcl10*, *Ccl5*, *Stat2*, and *Cxcl11* were down-regulated, rendering the DMSCs unable to recruit T cells and inhibiting T cell infiltration. In addition, we also found that the expression of *Serping1*, *C3*, *C2*, *C1s1*, *C1ra*, *C1s2*, and *Cfb* were down-regulated, thus inhibiting the complement activation pathway. Based on the above results, we believe that *Prx II* knockout can reduce the allograft rejection of DMSCs.

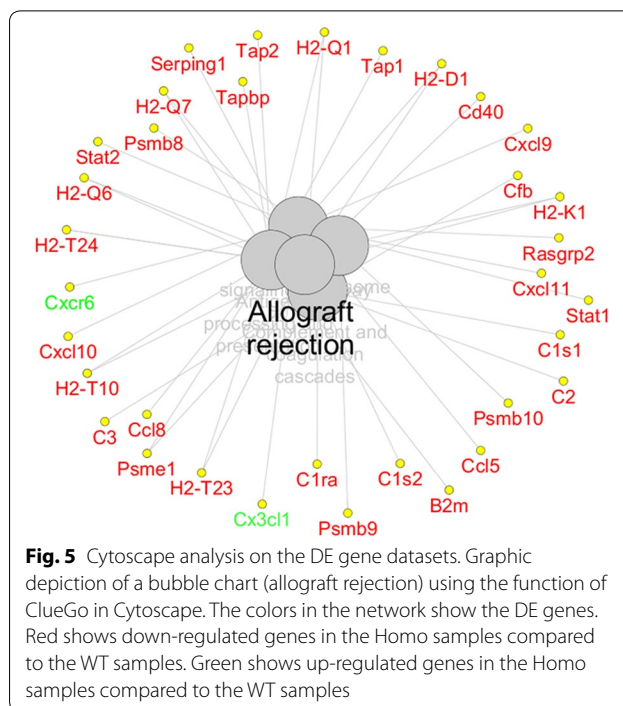
It has been shown that DMSCs do not express MHC-II molecules and present low expression of MHC-I molecules [30]. However, after allograft DMSCs were transplanted to recipients, immune rejection still took place, resulting in low survival rate and short survival

time [31]. In this study, we found that the DMSCs with *Prx II* knockout expressed lower MHC-I molecules and weakened T cell-specific immune responses. The specific regulatory mechanism needs further study.

In conclusion, immune rejection is the most difficult problem in the field of transplantation. Finding out how to induce the recipients to develop immune tolerance to the graft would be a turning point in this field. In this study, high-throughput sequencing technology shows that *Prx II* gene knockout may reduce immune rejection after DMSCs transplantation and prolong the survival time of DMSCs in vivo, providing a theoretical basis for allogeneic DMSC transplantation.

**Table 2 KEGG analysis of differentially expressed genes in PrxII<sup>+/+</sup> (WT) and PrxII<sup>-/-</sup>**

Path ID	Pathway	Count	p-value
mmu04612	Antigen processing and presentation	13	3.95E-11
mmu05160	Hepatitis C	13	3.04E-08
mmu04623	Cytosolic DNA-sensing pathway	9	4.74E-08
mmu05330	Allograft rejection	9	4.74E-08
mmu05332	Graft-versus-host disease	9	6.51E-08
mmu04940	Type I diabetes mellitus	9	1.37E-07
mmu05320	Autoimmune thyroid disease	9	4.46E-07
mmu05416	Viral myocarditis	9	3.99E-06
mmu04145	Phagosome	12	4.60E-06
mmu04620	Toll-like receptor signaling pathway	9	7.92E-06
mmu04622	RIG-I-like receptor signaling pathway	7	3.68E-05
mmu04514	Cell adhesion molecules (CAMs)	10	4.01E-05
mmu05150	Staphylococcus aureus infection	6	5.87E-05
mmu04610	Complement and coagulation cascades	7	6.91E-05
mmu04144	Endocytosis	11	1.97E-04
mmu03050	Proteasome	4	3.11E-03
mmu04062	Chemokine signaling pathway	8	3.99E-03
mmu05322	Systemic lupus erythematosus	7	4.44E-03
mmu04060	Cytokine-cytokine receptor interaction	9	5.96E-03
mmu04621	NOD-like receptor signaling pathway	4	7.74E-03
mmu05340	Primary immunodeficiency	3	1.07E-02
mmu00603	Glycosphingolipid biosynthesis—globo series	2	1.71E-02
mmu04380	Osteoclast differentiation	5	2.18E-02
mmu02010	ABC transporters	3	2.28E-02



**Table 3 Five signaling pathways related to allograft rejection were screened according to differentially expressed genes in PrxII<sup>+/+</sup> (WT) and PrxII<sup>-/-</sup>**

Pathway	Count	p-value	Down-regulated genes	Up-regulated genes
Allograft rejection	9	4.7387E-08	Cd40/H2-T24/H2-Q7/H2-K1/H2-T23/H2-Q6/H2-D1/H2-T10/H2-Q1	
Antigen processing and presentation	13	3.9502E-11	Psme1/Tapbp/Tap2/Tap1/H2-T24 /H2-Q7/B2m/H2-K1/H2-T23/H2-Q6/H2-D1/H2-T10/H2-Q1	
Proteasome	4	0.003105029	Psme1/Psmb8/Psmb10/Psmb9	
Chemokine signaling pathway	8	0.003985326	Ccl8/Stat1/Cxcl9/Rasgrp2/Cxcl10/Ccl5/Stat2/Cxcl11	Cx3cl1/Cxcr6
Complement and coagulation cascades	7	6.9125E-05	Serping1/C3/C2/C1s1/C1ra/C1s2/Cfb	



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### Authors' contributions

Y-HH, Y-YM and N-NY writing the manuscript, M-HJ, Y-HJ and G-NS, Y-QZ performed the experiments, A-GW, Y-DC, L-YY, Y-JJ, D-SL and H-NS performed the data analysis and funding supplying, Y-HH, JK and TK made substantial contributions to conception and design. All Authors read and approved the final article.

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### Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### References

- Kobolaj J, Dinnyes A, Memic A, Khademhosseini A, Mobasheri A (2016) Mesenchymal stem cells: identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. *Methods* 99:62–68
- D'souza N, Rossignoli F, Golinelli G, Grisendi G, Spano C, Candini O, Osturu S, Catani F, Paolucci P, Horwitz EM (2015) Mesenchymal stem/stromal cells as a delivery platform in cell and gene therapies. *BMC Med* 13(1):186
- Lu D-f, Yao Y, Su Z-z, Zeng Z-h, Xing X-w, He Z-y, Zhang C (2014) Down-regulation of HDAC1 is involved in the cardiomyocyte differentiation from mesenchymal stem cells in a myocardial microenvironment. *PLoS ONE* 9(4):e93222
- Watanabe S, Uchida K, Nakajima H, Matsuo H, Sugita D, Yoshida A, Honjoh K, Johnson WEB, Baba H (2015) Early transplantation of mesenchymal stem cells after spinal cord injury relieves pain hypersensitivity through suppression of pain-related signaling cascades and reduced inflammatory cell recruitment. *Stem Cells* 33(6):1902–1914
- Kato J, Kamiya H, Himeno T, Shibata T, Kondo M, Okawa T, Fujiya A, Fukami A, Uenishi E, Seino Y (2014) Mesenchymal stem cells ameliorate impaired wound healing through enhancing keratinocyte functions in diabetic foot ulcerations on the plantar skin of rats. *J Diabetes Complic* 28(5):588–595
- Vyas JM, Van der Veen AG, Ploegh HL (2008) The known unknowns of antigen processing and presentation. *Nat Rev Immunol* 8(8):607
- Sivanathan KN, Gronthos S, Rojas-Canales D, Thierry B, Coates PT (2014) Interferon-gamma modification of mesenchymal stem cells: implications of autologous and allogeneic mesenchymal stem cell therapy in allotransplantation. *Stem Cell Rev* 10(3):351–375
- Amadou C, Younger RM, Sims S, Matthews LH, Rogers J, Kumnovics A, Ziegler A, Beck S (2003) Fischer Lindahl K (2003) Co-duplication of olfactory receptor and MHC class I genes in the mouse major histocompatibility complex. *Hum Mol Genet* 12(22):3025–3040
- Vugmeyster Y, Glas R, Perarnau B, Lemonnier FA, Eisen H, Ploegh H (1998) Major histocompatibility complex (MHC) class I K<sub>b</sub>D<sub>b</sub>–/– deficient mice possess functional CD8+T cells and natural killer cells. *Proc Natl Acad Sci* 95(21):12492–12497
- Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med* 338(25):1813–1821
- Tan Y, Abdulreda MH, Cruz-Guilloty F, Cutrufello N, Shishido A, Martinez RE, Duffort S, Xia X, Echegaray-Mendez J, Levy RB (2013) Role of T cell recruitment and chemokine-regulated intra-graft T cell motility patterns in corneal allograft rejection. *Am J Transplant* 13(6):1461–1473
- Koga S, Auerbach MB, Engeman TM, Novick AC, Toma H, Fairchild RL (1999) T cell infiltration into class II MHC-disparate allografts and acute rejection is dependent on the IFN-gamma-induced chemokine Mig. *J Immunol* 163(9):4878–4885
- Fahy O, Porte H, Senechal S, Vorng H, McEuen AR, Buckley MG, Walls AF, Wallaert B, Tonnel AB, Tsicopoulos A (2001) Chemokine-induced cutaneous inflammatory cell infiltration in a model of Hu-PBMC-SCID mice grafted with human skin. *Am J Pathol* 158(3):1053–1063
- Horiguchi K, Kitagawa-Sakakida S, Sawa Y (2002) Selective chemokine and receptor gene expressions in allografts that develop transplant vasculopathy. *J Heart Lung Transplant* 21(10):1090–1100
- Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10(1):57
- Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman W-H, Pagès F, Trajanoski Z, Galon J (2009) ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25(8):1091–1093
- Monteiro BS, Neto NMA, Carlo RJD (2016) Mesenchymal stem cell infusion on skin wound healing of dexamethasone immunosuppressed wistar rats. *Ciência Rural* 40(1):238–245
- Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G (2012) The pleiotropic effects of erythropoietin in infection and inflammation. *Microbes Infect* 14(3):238–246
- Oku R, Oda S, Nakada T-a, Sadahiro T, Nakamura M, Hirayama Y, Abe R, Tateishi Y, Ito M, Iseki T (2013) Differential pattern of cell-surface and soluble TREM-1 between sepsis and SIRS. *Cytokine* 61(1):112–117
- Jirí Z, Michal F (2012) Changes in the serum levels of clusterin in children with sepsis. *Pediatr Pol* 88(1):6–13
- Eggenhofer E, Benseler V, Kroemer A, Popp F, Geissler E, Schlitt H, Baan C, Dahlke M, Hoogduijn MJ (2012) Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol* 3:297
- Dormady SP, Bashayan O, Dougherty R, Zhang X-M (2001) Basch RS (2001) Immortalized multipotential mesenchymal cells and the hematopoietic microenvironment. *J Hematother Stem Cell Res* 10(1):125–140
- Fox JM, Chamberlain G, Ashton BA (2007) Middleton J (2007) Recent advances into the understanding of mesenchymal stem cell trafficking. *Br J Haematol* 137(6):491–502
- Ishii T, Warabi E, Yanagawa T (2012) Novel roles of peroxiredoxins in inflammation, cancer and innate immunity. *J Clin Biochem Nutr* 50(2):91–105
- Geiben-Lynn R, Kursar M, Brown NV, Addo MM, Shau H, Lieberman J, Luster AD, Walker BD (2003) HIV-1 antiviral activity of recombinant natural killer cell enhancing factors, NKEF-A and NKEF-B, members of the peroxiredoxin family. *J Biol Chem* 278(3):1569–1574
- Asmal M, Letvin NL, Geiben-Lynn R (2013) Natural killer cell-dependent and non-dependent anti-viral activity of 2-Cys peroxiredoxin against HIV. *Int Trends Immun* 1(4):69–77
- Shau H, Gupta RK, Golub SH (1993) Identification of a natural killer enhancing factor (NKEF) from human erythroid cells. *Cell Immunol* 147(1):1–11

28. Yin G, Li C, Shan B, Wang W, Chen H, Zhong Y, Di J, Lin Q, Lin Y (2011) Insufficient peroxiredoxin-2 expression in uterine NK cells obtained from a murine model of abortion. *J Cell Biochem* 112(3):773–781
29. Sauri H, Ashjian PH, Kim AT, Shau H (1996) Recombinant natural killer enhancing factor augments natural killer cytotoxicity. *J Leukoc Biol* 59(6):925–931
30. Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD (2003) Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 10(2):228–241
31. Ding Y, Liang X, Zhang Y (2018) Rap1 deficiency-provoked paracrine dysfunction impairs immunosuppressive potency of mesenchymal stem cells in allograft rejection of heart transplantation. *Cell Death Dis* 9(3):386

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